

Synergistic effects of diuron and sedimentation on photosynthesis and survival of crustose coralline algae

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Abstract

Effects of short-term exposure to sedimentation and diuron, separately and in combination, on the photophysiology and survival of crustose coralline algae (CCA) were examined in controlled time-course experiments, using pulse-amplitude modulation (PAM) chlorophyll fluorometry. These experiments indicated that the effects of sediments and diuron, when applied in isolation, were often reversible, with recovery time dependant upon sediment type and diuron concentration. Exposure to fine (<63 µm grain size), nutrient-rich estuarine sediments reduced effective quantum yields ($\Delta F/F_m'$) of photosystem II in CCA species more than exposure to the same amount of fine (<63 µm grain size) calcareous sediments. Significant inhibition of photosynthesis ($\Delta F/F_m'$) was also observed at diuron concentrations $\geq 2.9 \mu\text{g L}^{-1}$. Fine estuarine sediments in combination with $\geq 0.79 \mu\text{g L}^{-1}$ dissolved diuron, caused yields ($\Delta F/F_m'$) to drop by 60% compared with controls after 24 h. The combined exposure to sediments and diuron also retarded recovery, thus $\Delta F/F_m'$ values were still only 60% of the controls after 9 days recovery in clean seawater. Mortality of CCA was observed in some fragments treated with combinations of sediment and diuron. Our results suggest that sediment deposition and exposure to diuron can negatively affect the photosynthetic activity of CCA, with sedimentation stress being significantly enhanced by the presence of trace concentrations of diuron.

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1. Introduction

1.1. Sedimentation on the Great Barrier Reef

Increasing sedimentation, resulting from anthropogenic sources, is a major concern for coastal coral reefs worldwide (Rogers, 1990; Hunte and Winttenberg, 1992; Dubinsky and Stambler, 1996; Wilkinson, 1996; McClanahan and Obura, 1997). In the Great Barrier Reef (GBR), an increase in soil erosion and sediment trans-

port since European settlement in the mid 1800's has lead to the prognosis of declining water quality (Haynes and Michalek-Wagner, 2000; Devlin et al., 2001; McCulloch et al., 2003). The consequences of sedimentation on reef organisms vary dramatically depending on grain size, exposure time and sediment composition (Rogers, 1983; Fabricius and Wolanski, 2000; Phillip and Fabricius, 2003). In general, sedimentation reduces rates of photosynthesis and enhances respiration and mucus production, which in turn negatively impacts growth rates and survival in some corals (Bak, 1978; Rogers, 1983; Riegl and Branch, 1995). A recent study has shown that some coral species are more sensitive to fine grained silt size sediments compared to coarser sediments (Weber, 2003). A large proportion of the

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sediment that is transported from catchments into the GBR lagoon consists of silt-sized particles, and this fine sediment has a greater potential to bind nutrients and biocides than coarse sediments (Kookana et al., 1998). The fine sediments are also easily transported and dispersed throughout the inner to mid-shelf regions of the GBR, representing a potential risk for approximately 740 reefs that occur in the inshore section of the GBR (Furnas and Brodie, 1996).

1.2. Herbicides on the Great Barrier Reef

The herbicide diuron (*N'*-(3,4-dichlorophenyl)-*N,N*-dimethylurea) is one of a group of phenylurea herbicides that act by inhibiting photosynthesis in plants and algae (Conrad et al., 1993; Cobb, 1992). Diuron is used worldwide both in the marine environment, as an antifouling agent, and in the terrestrial environment, as an agricultural herbicide (Hamilton and Haydon, 1996; Boxell et al., 2000; Thomas et al., 2000; Martinez et al., 2001). Diuron is used extensively in Queensland sugar growing regions which are situated mainly within 50 km of the coast and lie within 26 major drainage basins and river catchments from just south of Brisbane, north to Mossman (Hamilton and Haydon, 1996). Diuron is the most widely distributed herbicide in water and sediment samples at most sampling sites between Townsville and Cairns. Concentrations of up to $10 \mu\text{g kg}^{-1}$ were detected in subtidal sediments of the wet tropics (Townsville to Port Douglas), adjacent to the mouths of the Herbert and Johnston Rivers (Haynes et al., 2000a). Organisms living on some of the nearshore coral reefs of the GBR are regularly exposed to river plumes that may transport this herbicide, both in dissolved form and adsorbed to soil particles. Diuron has been shown to inhibit photosynthesis in seagrasses at concentrations as low as $0.1 \mu\text{g/L}$ (Haynes et al., 2000b; Ralph, 2000) and dinoflagellates in corals at $0.3 \mu\text{g/L}$ (Jones and Kerswell, 2003); however, little is known of the effects of diuron on crustose coralline algae (CCA).

1.3. Potential effects of runoff on crustose coralline algae

Crustose coralline algae are among the most abundant and widespread occupants of hard marine substrata in the photic zone worldwide (Steneck, 1986) and have long been assumed to be important factors in the processes of coral reef development and maintenance (Maudsley, 1990; Littler and Littler, 1997). Many CCA species induce larval settlement of numerous benthic invertebrates, including corals (Johnson and Sutton, 1994; Morse et al., 1988, 1996; Heyward and Negri, 1999), and provide refuge for invertebrates after metamorphosis. Accordingly, changes in CCA abundance can result in changes in structure and function of coral

reef ecosystems and can directly regulate the abundance of other components of reefs such as coral (Steneck and Testa, 1997).

The degree to which herbicides and increased sediment loads can affect abundance and species composition of CCA on reefs is poorly understood. Sediment has been shown to smother CCA, decreasing the survivorship of existing patches and preventing recruitment of CCA (Steneck, 1997; Figueiredo and Steneck, 2000; Fabricius and De'ath, 2001). Furthermore, the abundance of certain CCA species on the GBR was found to be negatively correlated to sediment deposition (Harrington, 2004). Flood events large enough to cause increased sediment transport onto nearshore reefs are also likely to convey herbicides such as diuron. However, no study has so far addressed the combined effects of sediments and herbicides. If phototrophs such as CCA are adversely affected by these flood plume conditions, then subsequent effects on coral communities that rely on CCA as recruitment substrata are also likely. In this laboratory study we exposed nearshore and offshore CCA species to sediments and diuron separately and in combination, and investigated the effects of such exposures on photosynthesis and survival of these organisms.

2. Materials and methods

Three species of CCA were subjected to sediments and one species to sediments and diuron individually and in combination in outdoor tanks. The primary responses of interest were altered photophysiology (quantified using PAM fluorometry), bleaching and survival.

2.1. CCA collection and preparation

Crustose coralline algae were collected from reef crests in the GBR and transported to the aquarium facilities at the Australian Institute of Marine Science (AIMS), where they were held in a flow-through 1000 L tank under a pivoted PVC trough that tipped ~ 25 L of seawater every ~ 2 min to mimic a high-energy environment. Shading was provided to reduce the amount of fouling.

For the preliminary experiments, one species of CCA, *Hydrolithon reinboldii* was collected from midshelf reefs along the Innisfail coastline (Lat. $17^{\circ}14'S$; Long. $146^{\circ}27'E$). For the sediment exposures, three CCA species, *H. reinboldii*, *Neogoniolithon fosliei* and *Porolithon onkodes*, were collected from midshelf reefs along the Innisfail coastline. For the combined sediment and diuron exposures, *P. onkodes* was collected from Davies Reef (Lat. $18^{\circ}50'S$; Long. $147^{\circ}742'E$).

Live samples of the specific CCA species for each experiment were prepared by carefully removing epi-

phytic organisms under flowing seawater. The CCA was cut into small fragments using a surgical bone cutter. Fragment thallus surface area was determined using the foil wrap technique (Marsh, 1970) and averaged 10.0 ± 0.4 (SE) cm^2 .

2.2. Sediment collection and preparation

Four types of sediments were used in the experiments. Estuarine sediment was collected from the upper 5 cm of a mud bank on the lower mangrove fringed Herbert River (Lat. $18^\circ 31'S$; Long. $146^\circ 19'E$). This sediment was sieved into two particle size fractions $<63\mu\text{m}$ (Herbert River fine silt, HRF) and $63\text{--}250\mu\text{m}$ (Herbert River medium fine sand, HRMF) as outlined in Table 1. Fine offshore sediments (OSF) were collected from Otter Reef (Lat. $18^\circ 03'S$; Long. $146^\circ 33'E$) and sieved to retrieve the $<63\mu\text{m}$ fraction. Fine calcareous sediment (KF) was prepared as sawdust by cutting dried skeletons of massive *Porites* corals (curtesy of Monty Devereux, AIMS), and also sieved to retrieve the $<63\mu\text{m}$ fraction.

2.3. Diuron stock preparation

A stock solution of diuron (Sigma, St. Louis, USA) was prepared prior to experimentation at a concentration of 10mg L^{-1} . Ethanol (EtOH) was used as a carrier to enhance the dissolution of diuron ($5\text{mL ethanol L}^{-1}$ stock), and stock solutions were then made up to a total volume of 1 L with GF/C (Whatman, Kent, UK) filtered seawater. A carrier control treatment was prepared with a second stock solution containing 5mL EtOH in filtered seawater.

2.4. Pulse amplitude modulated (PAM) fluorometry

Photosynthetic efficiency of the CCA species was determined by measuring variable chlorophyll fluorescence of photosystem II with a pulse amplitude modulated (PAM) chlorophyll fluorometer (Diving PAM,

Walz, Germany) (see Schreiber et al., 1986). PAM fluorometry has previously been used to assess the effects of herbicides on other groups of marine organisms (Ralph, 2000; Jones and Kerswell, 2003; Jones et al., 2003). Constant fluorescence (F) was determined in response to a weak pulse-modulated red measuring light ($0.15\mu\text{mol quanta m}^{-2}\text{s}^{-1}$). The maximum fluorescence (F_m) at 650nm was then measured by applying a saturating pulse of actinic light ($>3000\mu\text{mol quanta m}^{-2}\text{s}^{-1}$). A consistent distance of 3 mm between the fiber optic sensor (5.5mm diameter) and CCA surface was maintained using a black plastic spacer. Fifteen measurements, evenly distributed over the surface, were made on each CCA fragment. The maximum effective quantum yields of light-adapted CCA ($[F_m' - F]/F_m' = \Delta F/F_m'$) were taken outdoors over the specified time-course, in illuminated states when illumination was $\sim 50\mu\text{mol quanta m}^{-2}\text{s}^{-1}$. The CCA were exposed under partially shaded natural sunlight that reached up to $\sim 270\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ daily.

2.5. Preliminary studies

The maximum potential quantum yield is defined by the equation ($[F_m - F_0]/F_m$), where F_0 is the minimum fluorescence and F_m the maximum fluorescence in dark-adapted samples. Initial yield measurements revealed unusually low dark-adapted yields for CCA in comparison with higher plants, possibly due to a high minimum fluorescence (F_0) (Ting and Owens, 1992; Buchel and Wilhelm, 1993). The influence of measuring intensity on F_0 was tested for *H. reinboldii*. Two glass flow chambers (15 L) were used, each filled with 10 L GF/C filtered seawater. Three CCA fragments within each tank acted as replicates. The aerated chambers were arranged outdoors within a water bath to maintain a water temperature of 28°C with flow speed set to 5cm s^{-1} . A series of dark-adapted yield measurements were made on the CCA at a range of measuring light intensities (3–12) and modulation frequencies (0.6 kHz and 20 kHz). The sensitivity of CCA to light was assessed using the same experimental design. Simultaneous $\Delta F/F_m'$ and light intensity measurements were taken at 1 h intervals during the day under partially shaded conditions described in Section 2.4.

2.6. Sediment exposure

PAM fluorometry was used to quantify the photosynthetic stress in *H. reinboldii*, *N. fosliei* and *P. onkodes* in response to short-term exposures to the four sediment types at a deposition level of 103 ± 14 (SE) mg cm^{-2} (range: $77\text{--}116\text{mg cm}^{-2}$, Table 2).

Crustose coralline algae fragments were transferred to eight aquaria (25 L). Two replicate aquaria were used for each sediment type, each containing three replicate

Table 1

Contents of total nitrogen (N), total phosphorus (P) total organic carbon (OC) and diuron in four sediments types: HRMF is the Herbert River medium fine, HRF is the Herbert River fine, OSF is the offshore fine and KF is the calcareous fine (* from Weber, 2003)

Sediment types	Grain size* (μm)	N (%)*	P (%)*	OC (%)*	Diuron ($\mu\text{g kg}^{-1}$)	Diuron ($\mu\text{g kg}^{-1}$)
HRMF	63–250	0.019	0.009	0.529	0.20	0.095
HRF	<63	0.174	0.036	1.80	0.28	0.039
OSF	<63	0.153	0.033	0.153	0.16	0.27
KF	<63	0.032	0.004	0.173	BDL	–

Below detectable limit (BDL) $< 0.1\mu\text{g kg}^{-1}$. The potential water concentrations were calculated from the partitioning coefficient of diuron between organic carbon and water ($K_{oc} = 398$, see Haynes et al., 2000a).

Table 2
Treatments for combined sediment and diuron experiment

Treatment	30DS	10DS	3DS	1DS	S	30D	10D	3D	1D	EtOH	SW
Nominal diuron Conc. ($\mu\text{g L}^{-1}$)	30	10	3	1	0	30	10	3	1	0	0
Nominal sediment (HRF) deposition (mg cm^{-2})	96	96	96	96	96	0	0	0	0	0	0
Measured diuron conc. ($\mu\text{g L}^{-1}$)											
Time = 0	23.6	7.99	2.56	0.79	0	26.9	10.8	2.86	1.02	0	0
Time = 105	19	4.73	1.99	NA	0	22.4	4.92	2.13	0.571	0	0

Diuron concentrations were measured at beginning and end of exposure periods. Abbreviations: 30DS, 10DS, 3DS, 1DS = 30, 10, 3, 1 $\mu\text{g L}^{-1}$ diuron respectively, mixed with sediments. 30D, 10D, 3D, 1D = 30, 10, 3, 1 $\mu\text{g L}^{-1}$ diuron respectively. S is the sediments, EtOH = EtOH control, SW is the seawater control, NA = not measured.

CCA fragments per species, per time (nine fragments per species within each tank). The sediments were aerated for 48 h in a seawater container before being added to the experimental aquaria. Before addition of the sediment treatments, the control CCA fragments were temporarily transferred to a holding tank. The sediments were stirred within the experimental tanks to ensure uniform distribution onto the CCA surfaces. Four petri dishes were placed within each replicate aquarium. Once the sediments had settled after 12 h, the dishes were removed and the contents of each petri dish were dried and weighed to estimate sedimentation levels per unit area. When the sediment had settled the control fragments were returned to their designated experimental tank and evenly distributed between the now sediment loaded CCA fragments. Measured levels of sediment deposition were 96 ± 19 SE mg cm^{-2} for HRF, 96 ± 17 SE mg cm^{-2} for HRMF, 105 ± 9.6 SE mg cm^{-2} for OSF, and 96 ± 11 SE mg cm^{-2} for KF.

The aquaria were arranged outdoors under 70% shading within a water bath to maintain a water temperature of 28 °C. Small air stones within each aquarium provided gentle aeration. After the sediments had settled, flow-through seawater was provided for the duration of the exposure period. At the pre-nominated exposure times (See Fig. 1) three of the nine replicate sediment-loaded fragments per species were removed from the experimental aquaria. The sediments were gently washed from the surface of each fragment and effective photosystem II quantum yields ($\Delta F/F_m'$) were determined. The fragments were subsequently transferred back into identical sediment-free aquaria to examine recovery for up to 3.5 days.

2.7. Combined diuron and sediment exposure

In this experiment, PAM fluorometry was used to determine whether sedimentation stress is altered by the presence of diuron.

Diuron concentrations as high as $8.5 \mu\text{g L}^{-1}$ were reported in rivers feeding into the GBR (Mitchell et al., in press) and range-finding studies revealed that diuron in isolation did not inhibit photosynthesis at concentrations less than $1 \mu\text{g L}^{-1}$ (data not shown). Therefore, in

this experiment one type of sediment (HRF is the fine estuarine sediment) and four diuron concentrations (nominally 1, 3, 10 and $30 \mu\text{g L}^{-1}$) were applied in different treatments (Table 2). Fifteen replicate *P. onkodes* fragments were placed in eleven 15 L glass flow chambers, containing 10 L of GF/C filtered seawater. One chamber was used for each of the 11 treatments (Table 2). The CCA fragments were maintained in control conditions (seawater with and without EtOH) and exposed to nine-sediment/diuron treatments for up to 105 h under natural, 30% sunlight (up to $\sim 270 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$).

Diuron stock was added to aerated HRF sediments suspended in seawater 24 h prior to CCA exposure and these contaminated wet sediments were distributed within the water column of the experimental tanks to a final sediment level of 96 ± 19 (SE) mg cm^{-2} . Dissolved diuron concentrations were measured at the beginning and end of exposures (see Section 2.8). At the pre-nominated exposure times, three out of 15 replicate fragments of *P. onkodes* were removed from the experimental aquaria. The sediments were gently washed from the surface of each fragment and $\Delta F/F_m'$ was determined. Crustose coralline algae were subsequently transferred back into identical sediment- and contaminant-free aquaria to examine recovery for up to 9 days.

2.8. Extraction and analysis of diuron

Sediments (~ 50 g wet weight) were shaken overnight with acetone (AR, Merck, UK). Dichloromethane (AR, Merck, UK) was added to the sediment–acetone mixture, shaken well and then centrifuged. The supernatant was cleaned with saturated sodium chloride to remove extraneous material and the organic layer concentrated by rotary evaporation then restored in hexane (AR, Merck, UK) for LC/MS analysis. The extraction of seawater samples and herbicide analyses by liquid chromatography-mass spectrometry (HPLC-MS/MS) were performed as per Negri et al. (in press). The limits of detection for diuron were $\sim 0.1 \mu\text{g kg}^{-1}$ in sediment and $\sim 0.5 \mu\text{g L}^{-1}$ in seawater.

The seawater analyses revealed that diuron concentrations decreased over time both in the presence and

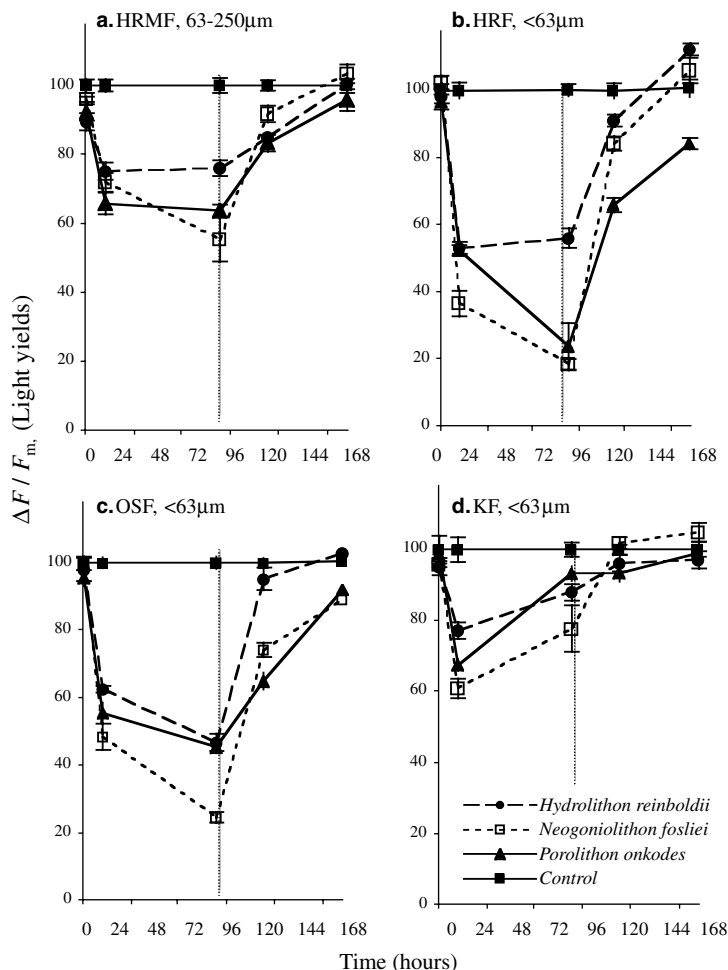


Fig. 1. Responses of photosynthetic yields in three species of CCA exposed to four sediment types: (a) Herbert River medium-fine (HRMF, 63–250μm), (b) Herbert River fine (HRF, <63μm), (c) Offshore fine (OSF, <63μm), (d) Calcareous fine (KF, <63μm). T 0h = before sediment application. T 84h = end of exposure, removal of all sediments, beginning of recovery. Dotted line separates exposure and recovery from treatments.

absence of sediment (Table 2); however, the nominal diuron concentrations (similar to initial exposure values) are shown throughout to simplify presentation.

2.9. Data treatment

Analyses of variance (ANOVA) were used to compare the effects of treatments, species and exposure times. Variances were tested for heterogeneity using Cochran's *C*-test and transformations were deemed unnecessary, therefore all analysis were performed on raw data. Significant effects were investigated further using Student Newman Keuls (SNK) tests, to determine which of the treatment levels were significantly different among treatments and species (Zar, 1999). All analyses were done with SPSS statistical software, Version 11. For graphical presentation, the light-adapted yields of photosystem II ($\Delta F/F_m'$) were expressed as a percentage of the pooled controls (filtered seawater with and without EtOH carrier) at the same time.

3. Results

3.1. Preliminary studies

3.1.1. Preliminary studies on dark-adapted yields of CCA

In the dark-adapted CCA, F_0 increased six fold between the two modulation frequencies at both the lowest (3) and highest (12) measuring light intensities tested (data not shown). This indicated that the measuring light of the PAM fluorometer was causing closure of reaction centres, leading to an increase in F_0 and subsequent decrease in yield. As a consequence the PAM fluorometer could not be used to obtain reliable values for F_0 for these CCA species. This phenomenon explained the low dark-adapted yields and meant that only the maximum effective quantum yields (light-adapted) could be interpreted reliably in subsequent experiments. Preliminary experiments also revealed that CCA colonies exhibited strong photoinhibition at irradiances above $200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Maximum $\Delta F/F_m'$ values were

observed at illumination levels less than $50 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. In remaining experiments, all CCA were partially shaded to avoid exposure to direct sunlight and yield measurements were taken at 8 AM daily to maintain similar illumination conditions.

3.2. Sediment exposure

In sediment-free seawater the three species, *H. reinboldii*, *N. fosliei* and *P. onkodes*, exhibited $\Delta F/F_m'$ values of 0.59 ± 0.10 , 0.62 ± 0.14 and 0.59 ± 0.14 respectively. There was no significant difference in these controls among the three species for the duration of the experiment (Table 3a).

There were significant differences in $\Delta F/F_m'$ between sediment treatments as well as between CCA species (Table 3b). *Hydrolithon reinboldii* was most tolerant to all sedimentation types (Table 4a). *Porolithon onkodes* and *N. fosliei* were both much less tolerant to sedimentation than *H. reinboldii* (Table 4a). After 84h, exposure to fine silt estuarine (HRF) and offshore (OSF) sediments reduced $\Delta F/F_m'$ more than exposure to the same amount of fine calcareous (KF) and coarse estuarine (HRMF) sediments (Table 4b). HRF and OSF sediments contained higher levels of phosphorous and nitrogen than HRMF and KF (Table 1). Herbicide analysis of the sediments revealed trace levels of diuron, with HRF containing slightly higher concentrations than the other samples (Table 1). The maximum concentration detected was $0.28 \mu\text{g kg}^{-1}$ in the HRF sample; however the level of dissolved diuron in subsequent treatments was not detectable (see Table 2). Post-hoc pairwise comparisons revealed there was no significant difference in responses between CCA exposed to the two nutrient-rich sediments, HRF and OSF, or between the two nutrient-poor sediment treatments, HRMF and KF. Responses did differ between the nutrient rich and nutrient poor sediments (Table 4b). The maximum $\Delta F/F_m'$

Table 3

Two way ANOVA testing for the effects of: (a) time on $\Delta F/F_m'$, in three species of CCA (*H. reinboldii*, *N. fosliei* and *P. onkodes*) at control conditions (without sediment) and (b) exposure to four sediment types on $\Delta F/F_m'$, in *H. reinboldii*, *N. fosliei* and *P. onkodes* after 84h exposure (Fig. 1)

	df	MS	F	p
a				
Species	2	0.0009	0.48	0.623
Time	5	0.053	2.6	0.093
Species:time	10	0.0021	1.15	0.34
Residuals	72	0.0018		
b				
Sediment	3	0.243	18.55	0.0001
Species	2	0.061	8.44	0.0009
Sediment:species	6	0.0088	1.09	0.3984
Residual	120			

Table 4

Post-hoc SNK results testing for differences in photosynthetic yields; (a) among three species of CCA exposed to four sediment types after 84h exposure, (b) among four sediment types, and (c) among three species of CCA exposed to four sediment types after 184h recovery from 84h exposure

a				
Mean	0.3395		0.2696	0.246
SE	0.0351		0.0469	0.0442
SNK	<i>H. reinboldii</i>	>	<i>N. fosliei</i>	<i>P. onkodes</i>
b				
Mean	0.4618	0.3836	0.1477	0.147
SE	0.0272	0.0261	0.0301	0.0294
SNK	KF	HRMF	>	HRF OS
c				
HRMF:	<i>H. reinboldii</i> =	<i>N. fosliei</i> =	<i>P. onkodes</i>	
HRF:	<i>H. reinboldii</i> =	<i>N. fosliei</i> >	<i>P. onkodes</i>	
Mean	0.603	0.5912	0.4816	
SE	0.019	0.017	0.014	
OS:	<i>H. reinboldii</i>	<i>N. fosliei</i>	<i>P. onkodes</i>	
KF:	<i>H. reinboldii</i> =	<i>N. fosliei</i> =	<i>P. onkodes</i>	

For SNK tests, the sample means were ranked and pairwise differences between means determined. Means and SE are untransformed data. Underlined means were not significantly different. Abbreviations: HRMF is the Herbert River 63–250 μm , HRF is the Herbert River <63 μm , OSF is the offshore fine <63 μm , KF is the calcareous fine <63 μm .

F_m' values of HRF and OSF fine sediment exposed CCA fragments declined to levels below 30% in *N. fosliei* and *P. onkodes* after 84h coverage, while fragments exposed to HRMF and KF remained above 50% and 60% respectively of the control values throughout the exposure period (Fig. 1).

The fragments were cleared of visible sediments and transferred to uncontaminated running seawater, and after 3.5 days all three species exposed to coarse estuarine (HRMF), fine offshore (OSF) and fine calcareous (KF) sediments had fully recovered their photosynthetic capacity (Table 4c). Both *H. reinboldii* and *N. fosliei* also recovered from exposure to fine silt estuarine (HRF) sediment after 3.5 days; however, *P. onkodes* only recovered to 80% of the controls. At this time, *P. onkodes* was significantly different from the other two species (ANOVA: $F_{(2,24)} = 6.26$, $p = 0.02$) and parts of the fragments showed loss of pigmentation. No mortality ($\Delta F/F_m'$ of zero) occurred after these treatments.

3.3. Combined diuron and sediment exposure

In this experiment, *P. onkodes* was subjected to diuron and sediment independently and in combination. The light-adapted yields $\Delta F/F_m'$ of *P. onkodes* for all fragments ranged from 0.52 to 0.61 at the beginning of the experiment.

Diuron alone inhibited photosynthesis at $2.9 \mu\text{g L}^{-1}$ (dissolved) following 9 and 35h exposure (Fig. 2a, Table

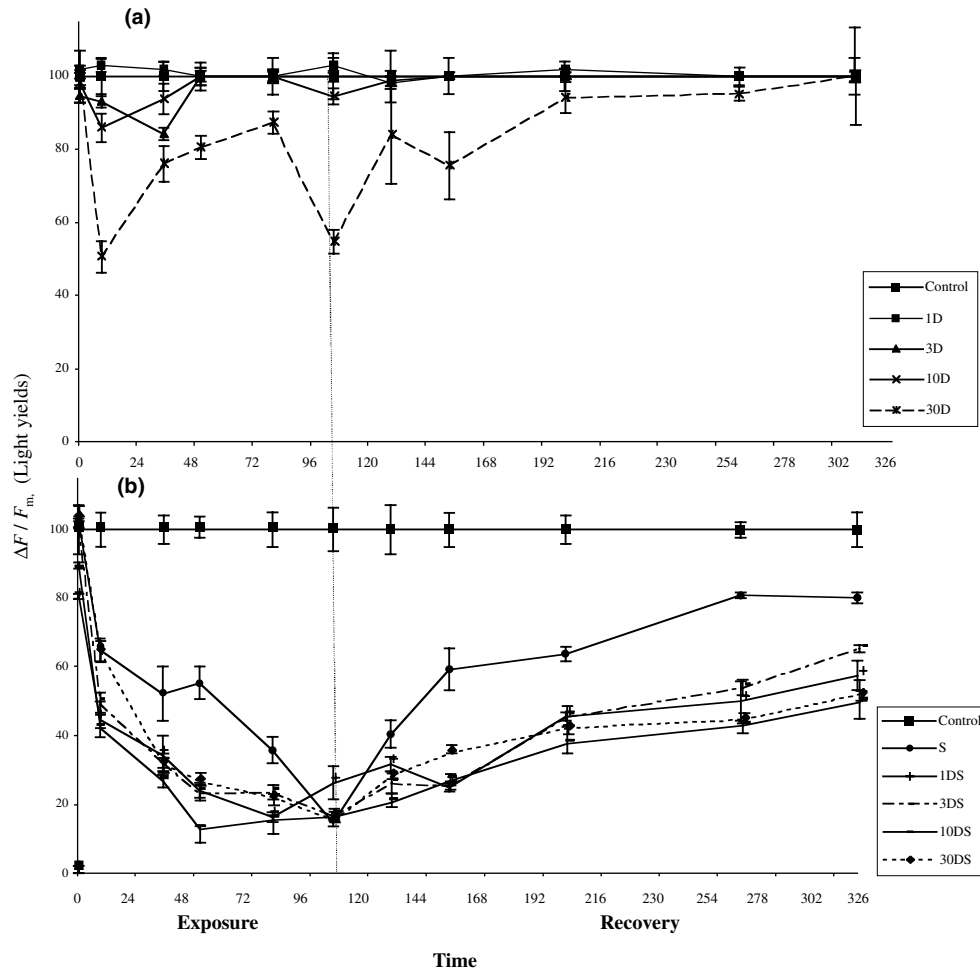


Fig. 2. Exposure and recovery of photosynthetic efficiency of *P. onkodes* exposed to a. diuron and b. sediments and diuron. For abbreviations see Table 2. Dotted line separates exposure and recovery from treatments.

2). The $\Delta F/F_m'$ values for most diuron-only treatments reverted back to those of the control fragments during the exposure period, possibly due to a loss of dissolved diuron in the water column as the contaminated seawater was not renewed daily (see Table 2). However, a decrease in $\Delta F/F_m'$ in response to $27 \mu\text{g L}^{-1}$ diuron was observed throughout the exposure period (Fig. 2a). Following transfer to uncontaminated seawater, the $\Delta F/F_m'$ values of fragments that had been exposed to lower diuron concentrations reverted rapidly back to those of the control fragments (Fig. 2a, Table 5). The CCA frag-

ments exposed to $27 \mu\text{g L}^{-1}$ diuron recovered to control values within four days in uncontaminated seawater.

Exposure of *P. onkodes* to uncontaminated sediment resulted in a steady decrease in $\Delta F/F_m'$ over the exposure period to as low as 20% of the control after 4 days (Fig. 2b). After 9 days recovery from sediment exposure, $\Delta F/F_m'$ values for *P. onkodes* were only 80% of the controls and these were significantly different from other treatments (Table 5, Fig. 2b).

Initially, exposure to sediments contaminated with diuron caused the greatest drop in $\Delta F/F_m'$. After 35h

Table 5

Post-hoc SNK results testing for differences in photosynthetic yields in *P. onkodes* in response to treatment (four diuron concentrations mixed with sediments, sediments without diuron, four diuron concentrations and control) after 105h exposure and 321h (9 days) recovery

Exposure (105 h)	<u>1DS</u>	<u>3DS</u>	<u>10DS</u>	<u>30DS</u>	S	<u>30D</u>	<u>10D</u>	<u>1D</u>	<u>3D</u>	<u>Control</u>
Recovery (321 h)	<u>1DS</u>	<u>3DS</u>	<u>10DS</u>	<u>30DS</u>	S	<u>10D</u>	<u>30D</u>	<u>1D</u>	<u>3D</u>	<u>Control</u>

For SNK tests, the sample means were ranked and pairwise differences between means determined. Underlined treatments were not significantly different. For abbreviations, see Table 2.

of exposure to fine estuarine sediments and $\geq 0.79 \mu\text{g L}^{-1}$ diuron, CCA fragment yields ($\Delta F/F_m'$) dropped to less than 30% of controls. However, by the end of the exposure period there was no significant difference between any of the sediment treatments (Table 5, Fig. 2b). Combined exposure to sediments contaminated with $\geq 0.79 \mu\text{g L}^{-1}$ diuron retarded recovery compared with uncontaminated sediment exposures (Table 5), and yields were still only 60% of the control after 9 days recovery in clean seawater. Fragments in the contaminated sediment exposures were again lightly covered by an even layer of sediment that resulted in anoxia in part of the CCA. Some fragments were considered dead (17% of exposed CCA fragments had $\Delta F/F_m'$ of zero), and others exhibited bleached portions (59% of exposed CCA fragments were partially bleached and exhibited low $\Delta F/F_m'$ values).

4. Discussion

Sediment deposition can negatively affect the photosynthetic activity of CCA and this stress can be significantly enhanced by the presence of the herbicide diuron. Stress occurred when CCA were exposed to environmentally relevant concentrations and durations of coastal sediments and diuron, but was minimal when CCA were exposed to sedimentation by fine clean calcium carbonate and coarser sediments. If the laboratory experiments are indicative of processes in the field, in-shore coralline algae exposed to terrestrial runoff could indeed be harmed by sediments and herbicides.

4.1. Application of PAM fluorometry to CCA

The precise determination and interpretation of the photosynthetic yields in CCA were confounded by the light intensity effects and the physical and physiological differences between higher plants and phycoblin containing algae. However, the exploratory experiments provided guidance to enable subsequent diuron/sediment exposure experiments to be conducted effectively. Photosynthetic efficiency in CCA was very sensitive to light intensity with medium light intensity $> 200 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ causing dramatic drops in $\Delta F/F_m'$. Some species of CCA are found in both sun-exposed and shade habitats (Steneck, 1986), suggesting that they have developed strategies to both minimize stress associated with exposure to high light energy (Beach et al., 1997) and to cope effectively under low light conditions. For example, *P. onkodes* responds to its light environment by rapid photoacclimation explaining its wide distribution in a variety of light environments (Payri et al., 2001). In the present study, the depression of $\Delta F/F_m'$ at medium light intensity

meant that comparisons between treatments were only practical at low light levels $\sim 50 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

A reliable F_0 could not be obtained for dark-adapted CCA due to closure of reaction centres (Buchel and Wilhelm, 1993; Ting and Owens, 1992), even at the lowest practical measuring intensity of three (any intensity lower than this resulted in very low signal to noise ratios). The pigmentation and antenna organization of CCA results in very different absorption features in comparison to higher plants (Buchel and Wilhelm, 1993) and this can lead to differences in excited energy transfer (Owens, 1986). These differences often result in very high F_0 values in relation to F_m leading to decreased F_v/F_m ratios in phycoblin containing alga compared to higher plants (Payri et al., 2001; Buchel and Wilhelm, 1993). These effects resulted in the present study being limited to an assessment of the effects of sediments and diuron on photosynthetic yields ($\Delta F/F_m'$) of light-adapted CCA.

4.2. Effects of sediments

Eutrophication and increased sedimentation are considered to be among the most significant factors contributing to coral reef degradation (Rogers, 1990; Wilkinson, 1996; Bryant et al., 1998). Reduced light levels from high turbidity, increased nutrients and smothering by accumulating particles all affect reef organisms in various ways (Rogers, 1990). Sediments have been shown to smother CCA by creating anoxic conditions that decrease their survivorship and recruitment (Steneck et al., 1997; Figueiredo and Steneck, 2000). Responses of CCA to sedimentation varied between CCA species and in response to sediment grain size and composition. Furthermore, the development of anoxia, which was observed during these experiments and in the field (L. Harrington, personal observation), may have been one of the major causes of the induced stress responses shown in this study.

All CCA species were more sensitive to fine-grained near- and off-shore silts than to sediments with coarser grain sizes or nutrient-poor calcareous sediments. Fine-grained silt allows less gas exchange, and has a greater potential to bind nutrients and biocides than coarse sediments (Kookana et al., 1998), is transported over long distances, and is easily re-suspended by wave action and thus undergoes many cycles of deposition and re-suspension. Our results revealed that two fine, nearshore and offshore sediments affected $\Delta F/F_m'$ to a greater extent than the coarse nearshore and fine calcium carbonate sediments with low nutrient content. Enhanced pigment loss (bleaching) was only observed for CCA fragments exposed to the fine sediments, correlating well with prolonged reductions in $\Delta F/F_m'$.

The differences between responses to fine, nearshore and offshore sediments compared with the medium near-

shore and fine calcareous sediments might be due to rates of gas and light penetration through the sediment layers, nutrient content and contamination. Fine, low nutrient calcium carbonate sediments allowed for a greater amount of light penetration (7.7%) compared to the fine, nutrient-enriched nearshore (0.058%) and offshore sediments (0.17%) (Weber, 2003). Crustose coralline algae however, have the ability to survive under a wide range of light regimes, using photoacclimation as a strategy to minimize stress from exposure to high (Beach et al., 1997) or low light (Littler et al., 1985). It is therefore unlikely that the different effects caused by the four sediment types were related to their light absorption properties. It is not known whether the effects of fine, nearshore and offshore sediments were more detrimental than those of calcareous sediments because of the higher organic and nutrient contents of the former group (Table 1). The nearshore sediments (HRF, HRMF) also contained slightly higher concentrations of diuron than offshore sediments (OSF); however, these concentrations were very low (Table 1). When converted to potential water column concentrations using partition coefficients (see Haynes et al., 2000a) no pattern emerged between $\Delta F/F_m'$ and the trace diuron concentrations observed. Fine, nearshore and offshore sediments both caused anoxia. It is assumed that oxygen depletion and development of hydrogen sulphide in silt sediments is primarily caused by reduced gas exchange. Our experiments showed rapid effects after exposure periods of hours to days, but longer exposures are likely to further differentiate sedimentation effects based on their grain sizes and composition (Dethier and Steneck, 2001; McClanahan et al., 2003).

The change in $\Delta F/F_m'$ in response to sediments clearly differed among CCA species. *Neogoniolithon fosliei* and *P. onkodes*, two species that are dominant on mid- and outer-shelf reefs but are rare or absent on inshore reefs, were more sensitive to sedimentation than the less abundant *H. reinboldii* that is more common inshore. It is likely that their differences in tolerance to sedimentation contribute to the observed differences in cross-shelf distribution. The differences in sediment tolerances among species may be attributed to contrasting morphologies. For example, some species of CCA shed their upper thallus layers (i.e. epithallial shedding) as means of disposing of functionally damaged cells (Keats et al., 1997). Even though both *N. fosliei* and *P. onkodes* are capable of shedding their upper thallus layers, they were unable to clear the fine silt sediment during the exposure period, despite provision of gentle flow in the tanks. Furthermore, *P. onkodes* was not observed shedding during recovery. The sloughing layers of *N. fosliei* were thick and exuded large quantities of mucous. Although $\Delta F/F_m'$ values returned to control levels during recovery, it is possible that sediments may impact other metabolic functions not revealed by PAM fluorometry. *Hydrolithon reinboldii* is highly mammillate and covered with pits and crevices, thus possesses a morphology that differs from that of the flat, smooth species *P. onkodes* and *N. fosliei*. Sediment was observed to settle less evenly on *H. reinboldii*, resting in the crevices and between notches, while much of the surface remained uncovered. As the PAM fluorometer integrates the fluorescence values across the 5.5 mm diameter area under the fiber optic sensor to derive photosynthetic yields, it is likely that these yields might be higher for irregular species in which some proportions of the surfaces remained sediment-free.

The sediment exposure experiments showed that CCA were able to recover over time and possibly compartmentalize sedimentation stress. In several cases when sediment was unevenly distributed the sediment-free patches did not exhibit any reduction in $\Delta F/F_m'$, while $\Delta F/F_m'$ declined on sediment-covered patches of the same CCA fragments. In field surveys of inshore reefs of the GBR, bleached patches of CCA are often observed in direct association with natural sedimentation (Harrington, 2004). Even if a proportion of the CCA colony is killed by sedimentation, remaining colony surfaces (e.g., vertical or prominent patches where sediment cannot accumulate) will remain physiologically unaffected and should continue to grow. Such compartmentalization needs to be quantified in future studies, as it may be an important survival mechanism for CCA inhabiting turbid environments.

The ability to withstand sedimentation varies greatly among CCA species. *Neogoniolithon fosliei* and *Lithothamnium prolifer* are not found in silty inshore environments on the GBR and may be sensitive to sedimentation (Harrington, 2004). Although some non-geniculate CCA cannot tolerate sediment-dominated environments, certain species appear to grow well in areas of heavy sedimentation. In particular, *N. strictum*, a heavily branched CCA found in the Caribbean, is uniquely suited to surviving under carbonate sediments (Steneck et al., 1997; Bosence, 1985). This species has unusually abundant thin-walled and multiple fused cells that may act as conduits for photosynthates through the thallus (Steneck, 1986). Such enhanced translocation of photosynthates may explain the unique ability of certain species to persist when much or all of its thallus is buried under sediments (Steneck et al., 1997). In this species, sedimentation seemed to trigger lateral growth, which may enhance its chances of persisting in areas periodically uncovered by sediment. Even though some species have the ability to withstand certain types of sedimentation (i.e. carbonate sediments), most CCA species, including *N. strictum*, cannot recruit to sediment covered surfaces, as CCA spores require hard, relatively sediment-free substrata for successful germination and growth (Steneck et al., 1997).

4.3. Effects of diuron

Diuron, like 50% of the other commercially available herbicides, inhibits photosynthesis in marine organisms (Ralph, 2000; Jones and Kerswell, 2003; Jones et al., 2003; Owen et al., 2003). This evaluation of the effects of diuron on CCA indicates a significant reduction in the photosynthetic efficiency ($\Delta F/F_m'$) due to the reversible binding of the herbicide to the D1 protein in the chloroplast (Jones et al., 2003). Diuron caused a reduction in $\Delta F/F_m'$ at or above an exposure concentration of $2.9 \mu\text{g L}^{-1}$ after 9 h. This concentration is greater than the lowest observed effect concentration (LOEC) for symbiotic dinoflagellates in corals of $0.3 \mu\text{g L}^{-1}$ (Jones and Kerswell, 2003) and for seagrasses of $0.1 \mu\text{g L}^{-1}$ (Haynes et al., 2000b; Ralph, 2000). At higher diuron concentrations ($11\text{--}27 \mu\text{g L}^{-1}$) inhibition of $\Delta F/F_m'$ was also less pronounced in CCA than was reported for corals and seagrasses. It is possible that the uptake of diuron is slower across the multiple and heavily calcified vegetative tissue layers of CCA before it reaches the chloroplast target sites compared with the rapid transport to chloroplasts in other organisms. Furthermore, variation in rates of herbicide uptake may also depend on species-specific differences in cell permeability (Fahl et al., 1995). The reduction in $\Delta F/F_m'$ indicated impaired photosynthetic function but this effect was fully reversible when CCA fragments were transferred to uncontaminated seawater.

At high diuron concentrations ($29 \mu\text{g L}^{-1}$) visible bleaching (pigment loss and/or cell death) of CCA was observed and this again correlated with a long-term reduction in $\Delta F/F_m'$. Similar concentrations of diuron cause bleaching in corals due to the expulsion of symbiotic dinoflagellates (Negri et al., in press). Herbicide mediated bleaching in CCA however, is likely to be caused by the destruction of chloroplasts and carotenoids (or inhibition of their formation), which leads to lightening in coloration. The improvement of $\Delta F/F_m'$ values in the diuron exposed CCA following transfer to uncontaminated seawater can be attributed to reversible binding to the D1 protein, as well as repair processes within photosystem II and to the shedding of damaged cells with the lower yields.

4.4. Sediments contaminated with diuron: synergistic effects

Although all short-term effects of diuron and sedimentation in isolation were reversible under experimental conditions, the combination of diuron and sedimentation appeared to induce irreversible damage to photosystem II, subsequent bleaching and even death of the CCA. Each of the sediment exposures in this final experiment resulted in a similar decline in photosynthetic yields after 105 h exposure. Crustose coralline

algae exposed to uncontaminated sediments were able to recuperate to 80% of control $\Delta F/F_m'$ values by the end of the recovery period, whereas the CCA exposed to diuron-contaminated sediments recovered to less than 50% of the control $\Delta F/F_m'$ value in the same period. Simultaneous exposure to sedimentation and herbicides such as diuron is an environmentally relevant scenario adjacent to some catchments of the GBR, as the principal mode of non-ionic and organic pesticide transport from farms is through attachment to soil particles (Kookana et al., 1998). The exposure of nearshore marine organisms to herbicides is therefore most likely to occur in the presence of fine nearshore sediments. Furthermore herbicides tend to be more persistent within sediments than in the open water (Voulvoulis et al., 2002). Our experiment suggests that synergistic exposure to fine nutrient-rich nearshore sediments with adsorbed herbicides could pose a significant problem to benthic phototrophs of the GBR.

4.5. Environmental relevance—impacts on coral reef ecosystems

Numerous reefs around the world experience sedimentation rates in excess of $200 \text{ mg cm}^{-2} \text{ day}^{-1}$ for periods of days (Maragos, 1972; Mapstone et al., 1989; Riegl and Branch, 1995). In the central GBR, river plumes affect large proportions of coastal coral reefs, carrying on average between 5 and 50 mg L^{-1} of fine, organically and nutrient enriched suspended sediments with some concentrations reaching as high as 300 mg L^{-1} close to river mouths (Devlin et al., 2001). The maximum concentration of diuron detected in rivers flowing into the GBR is $8.5 \mu\text{g L}^{-1}$ (White et al., 2002; Mitchell et al., in press). Although concentrations on the near-shore reefs have not been measured, partitioning co-efficients and sediment concentrations have been used to estimate maximum water concentrations of between 0.1 and $1 \mu\text{g L}^{-1}$ in coastal environments (Haynes et al., 2000a). However, marine organisms rarely encounter only a single stressor, and multiple stressors pose significant effects to recruitment and the regenerative processes of marine assemblages (Hughes and Connell, 1999). In the present experiments, CCA was shown to be sensitive to environmentally relevant combinations of sediment (up to 116 mg cm^{-2}) and diuron ($\geq 0.79 \mu\text{g L}^{-1}$).

The resilience and perseverance of coral reefs that are faced with both natural and ever increasing anthropogenic disturbances, ultimately depends on the successful settlement and survival of reef dwelling corals. Coral recruitment is directly vulnerable to the effects of sedimentation (Birkeland, 1977; Richmond, 1993; Babcock and Smith, 2002) and pollution (Negri and Heyward, 2000, 2001). Here we have shown that the indirect effects of sediments and pollution on reef substrata also have

the potential to indirectly affect reef recovery and regeneration. Crustose coralline algae is thought to play a key role in facilitating coral recruitment (Richmond, 1997; Morse et al., 1988, 1996; Heyward and Negri, 1999), therefore any disturbance to CCA can have subsequent effects on the resilience of coral reefs. Gilmour (1999) demonstrated that sediment deposition on CCA forms a barrier that reduced coral larvae settlement. Other studies have shown that live CCA is significantly more effective in inducing coral larvae to settle than dead CCA (Harrington et al., in press). The present research represents the first attempt to understand the effects of environmentally relevant combinations of sediments and herbicides on critically important algae species. The synergistic effects of multiple stressors on the physiological health and survival of CCA demonstrated in this study highlight the need to examine the extent to which pollutants from terrestrial runoff can enhance the damaging effects of sediments and nutrients on coral reef ecosystems.

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